

WHAT IS CLAIMED IS:

1. A method of screening a composition for G protein-coupled receptor (GPCR) agonist activity comprising the steps of:

5 (a) providing a mixture of cells comprising at least a first cell and a second cell, the first cell comprising a first GPCR and a first conjugate of a first marker molecule and an arrestin protein, the second cell comprising a second GPCR different from the first GPCR and a second conjugate of a second marker molecule and an arrestin protein, the second conjugate being the same or different from the first conjugate;

10 (b) exposing the mixture of cells to a test composition; and

(c) determining, through detection of the marker molecules in the first and second conjugates, whether or not the composition gives an indication of GPCR agonist activity with respect to the first or second GPCRs.

15 2. The method of claim 1, wherein the marker molecules of the first and second conjugates are the same.

3. The method of claim 1, wherein the marker molecules of the first and second conjugates are different.

20 4. The method of claim 2, wherein the marker molecules of the first and second conjugates are green fluorescent protein.

5. The method of claim 1, wherein the first and second conjugates are the same.

25 6. The method of claim 1, wherein the marker molecule of the first conjugate and the marker molecule of the second conjugate are independently selected from the group consisting of radioisotope, epitope tag, affinity label, enzyme, fluorescent group, and chemiluminescent group.

30 7. The method of claim 1, wherein step (c) comprises detecting for translocation or localization of the first and second conjugates, detection of translocation or localization of the first or second conjugates being an indication that the composition has GPCR agonist activity.

8. The method of claim 1, wherein step (c) comprises detecting for translocation or localization of the first and second conjugates, an increase in translocation or localization of the first or second conjugates after exposure to the test composition being an indication that the composition has GPCR agonist activity.

9. The method of claim 1, wherein step (c) comprises detecting for translocation or localization of the first and second conjugates, an increased level of translocation or localization of the first or second conjugates with respect to a predetermined level of translocation or localization being an indication that the composition has GPCR agonist activity.

10. The method of claim 7, wherein the marker molecules of the first and second conjugates are the same and wherein the marker molecules of the first and second conjugates are green fluorescent protein.

11. The method of claim 1, wherein the first GPCR is stably expressed in the first cell and the second GPCR is stably expressed in the second cell.

12. The method of claim 1, wherein the first GPCR is transiently expressed in the first cell and the second GPCR is transiently expressed in the second cell.

13. The method of claim 1, wherein the first and second GPCRs interact with different G_α protein subunits.

14. A method of screening a composition for G protein-coupled receptor (GPCR) agonist activity comprising the steps of:

(a) providing a cell comprising a first GPCR, a second GPCR different from the first GPCR, a first conjugate of a marker molecule and an arrestin protein associated with the desensitization pathway of the first GPCR, and a second conjugate of a marker molecule and an arrestin protein associated with the desensitization pathway of the second GPCR, the second conjugate being the same or different from the first conjugate;

(b) exposing the cell to a test composition; and

(c) determining, through detection of the marker molecules in the first and second conjugates, whether or not the composition gives an indication of GPCR agonist activity with respect to the first or second GPCRs.

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15. The method of claim 14, wherein the first and second conjugates are the same.

16. The method of claim 15, wherein the marker molecules of the first and second conjugates are green fluorescent protein.

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17. The method of claim 14, wherein the marker molecule of the first conjugate and the marker molecule of the second conjugate are independently selected from the group consisting of radioisotope, epitope tag, affinity label, enzyme, fluorescent group, and chemiluminescent group.

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18. The method of claim 14, wherein step (c) comprises detecting for translocation or localization of the first and second conjugates, detection of translocation or localization of the first or second conjugates being an indication that the composition has GPCR agonist activity.

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19. The method of claim 14, wherein step (c) comprises detecting for translocation or localization of the first and second conjugates, an increase in translocation or localization of the first or second conjugates after exposure to the test composition being an indication that the composition has GPCR agonist activity.

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20. The method of claim 14, wherein step (c) comprises detecting for translocation or localization of the first and second conjugates, an increased level of translocation or localization of the first or second conjugates with respect to a predetermined level of translocation or localization being an indication that the composition has GPCR agonist activity.

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21. The method of claim 18, wherein the marker molecules of the first and second conjugates are the same and wherein the marker molecules of the first and second conjugates are green fluorescent protein.

22. The method of claim 14, wherein the first and second GPCRs are stably expressed in the cell.

5 23. The method of claim 14, wherein the first and second GPCRs are transiently expressed in the cell.

24. The method of claim 14, wherein the first and second GPCRs interact with different G_α protein subunits.

10 25. A method of screening a composition for G protein-coupled receptor (GPCR) agonist activity comprising the steps of:

(a) providing a mixture of cells comprising at least a first cell and a second cell, the first cell comprising a first GPCR conjugated to a first marker molecule, the second cell
15 comprising a second GPCR that is different from the first GPCR and is conjugated to a second marker molecule, the second marker molecule being the same or different from the first marker molecule;

(b) exposing the mixture of cells to a test composition; and

(c) determining, through detection of the first and second marker molecules, whether
20 or not the composition gives an indication of GPCR agonist activity with respect to the first or second GPCRs.

26. The method of claim 25, wherein the first and second marker molecules are the same.

25 27. The method of claim 25, wherein the first and second marker molecules are different.

28. The method of claim 26, wherein the first and second marker molecules are green
30 fluorescent protein.

29. The method of claim 25, wherein the first and second marker molecules are independently selected from the group consisting of radioisotope, epitope tag, affinity label,

enzyme, fluorescent group, and chemiluminescent group.

30. The method of claim 25, wherein step (c) comprises detecting for translocation or localization of the first and second marker molecules, detection of translocation or
5 localization of the first or second marker molecules being an indication that the composition has GPCR agonist activity.

31. The method of claim 25, wherein step (c) comprises detecting for translocation or localization of the first and second marker molecules, an increase in translocation or
10 localization of the first or second marker molecules after exposure to the test composition being an indication that the composition has GPCR agonist activity.

32. The method of claim 25, wherein step (c) comprises detecting for translocation or localization of the first and second marker molecules, an increased level of translocation or
15 localization of the first or second marker molecules with respect to a predetermined level of translocation or localization being an indication that the composition has GPCR agonist activity.

33. The method of claim 30, wherein the first and second marker molecules are green
20 fluorescent protein.

34. The method of claim 25, wherein the first GPCR conjugated to the first marker molecule is stably expressed in the first cell and the second GPCR conjugated to the second marker molecule is stably expressed in the second cell.

25 35. The method of claim 25, wherein the first GPCR conjugated to the first marker molecule is transiently expressed in the first cell and the second GPCR conjugated to the second marker molecule is transiently expressed in the second cell.

30 36. The method of claim 25, wherein the first and second GPCRs interact with different G_α protein subunits.

37. A method of screening a composition for G protein-coupled receptor (GPCR)

agonist activity comprising the steps of:

(a) providing a cell comprising a first GPCR conjugated to a first marker molecule and a second GPCR that is different from the first GPCR and is conjugated to a second marker molecule, the second marker molecule being the same or different
5 from the first marker molecule;

(b) exposing the cell to a test composition; and

(c) determining, through detection of the first and second marker molecules, whether or not the composition gives an indication of GPCR agonist activity with respect to the first or second GPCRs.

10 38. The method of claim 37, wherein the first and second marker molecules are the same.

15 39. The method of claim 38, wherein the first and second marker molecules are green fluorescent protein.

20 40. The method of claim 37, wherein the first and second marker molecules are independently selected from the group consisting of radioisotope, epitope tag, affinity label, enzyme, fluorescent group, and chemiluminescent group.

25 41. The method of claim 37, wherein step (c) comprises detecting for translocation or localization of the first and second marker molecules, detection of translocation or localization of the first or second marker molecules being an indication that the composition has GPCR agonist activity.

30 42. The method of claim 37, wherein step (c) comprises detecting for translocation or localization of the first and second marker molecules, an increase in translocation or localization of the first or second marker molecules after exposure to the test composition being an indication that the composition has GPCR agonist activity.

43. The method of claim 37, wherein step (c) comprises detecting for translocation or localization of the first and second marker molecules, an increased level of translocation or localization of the first or second marker molecules with respect to a predetermined level of translocation or localization being an indication that the composition has GPCR agonist

activity.

44. The method of claim 41, wherein the first and second marker molecules are green fluorescent protein.

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45. The method of claim 37, wherein the first GPCR conjugated to the first marker molecule and the second GPCR conjugated to the second marker molecule are stably expressed in the cell.

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46. The method of claim 37, wherein the first GPCR conjugated to the first marker molecule and the second GPCR conjugated to the second marker molecule are transiently expressed in the cell.

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47. The method of claim 37, wherein the first and second GPCRs interact with different G_α protein subunits.